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## **Double-blind, Randomized, 8-week Placebo-controlled followed by a 16-week open label extension study, with the LPA1 receptor antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis**

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**Abstract:** BACKGROUND Preclinical studies suggest a role for lysophosphatidic acid (LPA) in the pathogenesis of systemic sclerosis (SSc). OBJECTIVES SAR100842, a potent selective oral antagonist of LPA1 receptor, was assessed for safety, biomarkers and clinical efficacy in patients with diffuse cutaneous SSc (dcSSc). METHODS An 8-week double-blind, randomized, placebo-controlled study followed by a 16-week open label extension with SAR100842 was performed in patients with early dcSSc and a baseline Rodnan skin score (mRSS) of at least 15. The primary endpoint was safety during the double-blind phase of the trial. Exploratory endpoints included the identification of a LPA-induced gene signature in patients' skin. RESULTS 17 of 32 subjects were randomized to placebo and 15 to SAR100842; 30 patients participated in the extension study. The most frequent adverse events reported for SAR100842 during the blinded phase were headache, diarrhea, nausea and fall and the safety profile was acceptable during the extension part. At Week 8, mean reduction in mRSS was numerically greater in the SAR100842 compared to placebo (mean change [SD]: -3.57 [4.18] versus -2.76 [4.85]; difference [95% CI]: -1.2 [-4.37 to 2.02], p=0.46). A greater reduction of LPA related genes was observed in skin of SAR100842 group at Week 8, indicating LPA target engagement. CONCLUSION SAR100842, a selective orally available LPA receptor antagonist, was well tolerated in patients with dcSSc. MRSS improved during the study although not reaching significance, and additional gene signature analysis suggested target engagement. These results need to be confirmed in a larger controlled trial. This article is protected by copyright. All rights reserved.

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**Double-blind, Randomized, 8-week Placebo-controlled followed by a 16-week open label extension study, with the LPA1 receptor antagonist SAR100842 for Patients With Diffuse Cutaneous Systemic Sclerosis**

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**Jagerschmidt A, Illiano S, Ledein L, Boitier E and Agueusop I** are Sanofi's employees.

*Background:* Preclinical studies suggest a role for lysophosphatidic acid (LPA) in the pathogenesis of systemic sclerosis (SSc).

*Objectives:* SAR100842, a potent selective oral antagonist of LPA1 receptor, was assessed for safety, biomarkers and clinical efficacy in patients with diffuse cutaneous SSc (dcSSc).

*Methods:* An 8-week double-blind, randomized, placebo-controlled study followed by a 16-week open label extension with SAR100842 was performed in patients with early dcSSc and a baseline Rodnan skin score (mRSS) of at least 15. The primary endpoint was safety during the double-blind phase of the trial. Exploratory endpoints included the identification of a LPA-induced gene signature in patients' skin.

*Results:* 17 of 32 subjects were randomized to placebo and 15 to SAR100842; 30 patients participated in the extension study. The most frequent adverse events reported for SAR100842 during the blinded phase were headache, diarrhea, nausea and fall and the safety profile was acceptable during the extension part. At Week 8, mean reduction in mRSS was numerically greater in the SAR100842 compared to placebo (mean change [SD]: -3.57 [4.18] versus -2.76 [4.85]; difference [95% CI]: -1.2 [-4.37 to 2.02],  $p=0.46$ ). A greater reduction of LPA related genes was observed in skin of SAR100842 group at Week 8, indicating LPA<sub>1</sub> target engagement.

*Conclusion:* SAR100842, a selective orally available LPA<sub>1</sub> receptor antagonist, was well tolerated in patients with dcSSc. MRSS improved during the study although not reaching significance, and additional gene signature analysis suggested target engagement. These results need to be confirmed in a larger controlled trial.

**Clinicaltrials.gov** NCT01651143

Systemic sclerosis (SSc) is characterized by fibrosis of the skin and internal organs, prominent alterations of the microvasculature, and frequent abnormalities of cellular and humoral immunity (1). SSc is an orphan disease, with high morbidity, which strongly impairs the quality of life and has a high case-specific mortality (2). The high burden of severe skin and internal organ involvement in the early stages of diffuse cutaneous SSc (dcSSc) has been highlighted by many cohort studies. Safe and effective treatments for skin and other manifestations of dcSSc are lacking (3).

The pathogenesis of SSc is complex and at present there is no unifying theory that may explain all its aspects. Consensual models of pathogenesis have suggested that early vascular events associated with autoimmunity and inflammation lead to fibroblast activation and differentiation, promoting subsequent fibrosis. A broad range of biological processes interact in SSc and these include involvement of key profibrotic cytokines and growth factors, an imbalance in Th1/Th2/Th17/Treg systems promoting inflammation and fibrosis and activation of B cells promoting production of autoantibodies (1).

Lysophosphatidic acid (LPA) is a lipid mediator that signals through specific G-protein-coupled receptors, designated as LPA1 to LPA6. It is generated at sites of inflammation or cell injury by the action of lysophospholipase D (lysoPLD), also known as autotaxin, on lysophosphatidylcholine and other lysophospholipids (4). LPA exerts various physiological effects on the receptors of parenchymal cells with some tissue specificities with regards to the various receptors (5, 6, 7). LPA mediates a variety of cell activities, including mitogenesis, cell differentiation, cell survival, cytoskeletal reorganization, cell migration and extracellular matrix production. Recent studies looking at circulating markers, in vitro cell activation, or animal models have suggested that LPA is involved, and plays an important role, in the pathogenesis of SSc. The role of LPA has also been demonstrated in several animal models of organ fibrosis independently of SSc (8-11).

SAR100842 is a potent selective LPA1 receptor antagonist (Sanofi R&D, France). In vivo, SAR100842 reversed dermal thickening and significantly inhibited myofibroblast differentiation and collagen content in mouse skin fibrosis model. Similar anti-fibrotic properties were observed using the Tsk-1 mouse model (*Illiano et al, submitted*). Mechanistic investigations showed that the anti-fibrotic effects of LPA1 blockade could be mediated partly via inhibition of the Wnt signaling pathway.

Taking into account the promise of LPA<sub>1</sub> receptor blockade in fibrotic pre-clinical models and the unmet need of early dcSSc, we performed a randomized proof-of-biological activity study assessing the effects of SAR100842 in early dcSSc patients.

## PATIENTS AND METHODS

### Study design

This was a double-blind, randomized, placebo-controlled, 8-week Phase 2a study, followed with an open label extension for 16 weeks (Supplemental Figure 1).

The objective was to investigate the effects of orally administered SAR100842 in patients with dcSSc, to characterize safety, plasma pharmacokinetics (PK) and pharmacodynamics (PD) with a focus on clinical efficacy and on SSc related biomarkers. In the double-blind phase of the study, SAR100842 300 mg (100 mg + 200 mg tablets for a total daily dose of 600 mg) or matching placebo were administered orally twice a day.

Following a screening period of up to 14 days, eligible patients were randomized. Clinical and biological parameters were assessed and skin biopsies were taken at a pre-defined area of the forearm at baseline and end of treatment (Week 8).

Patients who had completed the 8-week treatment and who did not meet any discontinuation criterion (see supplement) were invited to participate in the open label non-controlled 16-week extension phase of the study with the same dosage of SAR100842 as in the initial part of the trial.

Patients were evaluated at the end of the extension part (Week 24) for clinical and biological assessments including two additional skin biopsies in consenting patients.

The dose of 300 mg BID was selected for the study based on activity/efficacy data from *in vitro* pharmacology models and *in vivo* animal disease models, as well as the safety profile observed in healthy volunteers (unpublished data).

The duration of 8-week was chosen based on expert opinion, suggesting that an 8-week treatment duration would be sufficient to demonstrate significant changes in SSc-related biomarkers. This design reduced the exposure of dcSSc patients to an experimental drug in this phase 2a study, while it provided the necessary data on safety and activity to support a full development in this indication.

A total of 12 active clinical sites located in Switzerland, France, UK, Italy, and USA participated in this study.

## **Patients**

Patients met the 1980 American College of Rheumatology (ACR) classification criteria for SSc, with diffuse cutaneous involvement (12), and had less than 36-month disease duration since the onset of first SSc manifestation other than Raynaud's phenomenon. A baseline modified Rodnan skin score (mRSS)  $\geq 15/51$  together with an area of definite involvement of the mid-volar forearm allowing 4 mm skin biopsies were other key inclusion criteria.

Immunosuppressive therapies stable for 4 weeks prior to enrollment were permitted including prednisolone up to 10 mg/d, methotrexate up to 25 mg/week, azathioprine up to 100 mg/d and mycophenolate mofetil up to 2 g daily (*see protocol in supplement for definition*).



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Patients experiencing orthostatic hypotension (postural reduction of systolic blood pressure by >20 mm Hg or reduction of diastolic blood pressure by >10 mm Hg), moderate to severe postural dizziness, pre-syncope or syncope within the last 6 months of screening were excluded, related to the current knowledge of the study drug obtained in phase 1 studies.

### Study endpoints

The primary endpoint was safety and tolerability during the 8-week treatment period. Secondary endpoints were: change from baseline to Week 8 in skin and blood biomarkers, changes from baseline to Week 8 in the mRSS and SHAQ, safety and tolerability during the extension treatment period and pharmacokinetics. Skin biopsies were used for RNA extraction and some mRNA biomarkers were assessed using quantitative PCR including: cartilage oligomeric matrix protein (COMP), thrombospondin 1 (THBS1), Plasminogen Activator Inhibitor type 1 (PAI-1), Wingless-Type MMTV integration site family member 2 (Wnt2) and secreted frizzled-related protein 4 (SFRP4). Other skin biopsies were dedicated to immunohistochemistry (IHC). Labeling for  $\alpha$ -SMA was performed on serial slides and skin thickness (histology) was evaluated. LPA markers were selected based on literature data and internal confirmation using dermal fibroblasts from SSc patients treated with LPA. The choice of other markers (COMP, THBS1, Col1 and  $\alpha$ SMA) was based on literature data selecting genes or proteins that may play a key role in the evolution of fibrosis in SSc patients (13, 14).

To explore the effect of SAR100842 on the LPA pathway, we used the results of a parallel study performed using cultured dermal fibroblasts from patients with SSc. LPA gene expression response was defined in the cultured dermal fibroblasts study. This LPA response was used in combination with the expression profile in patient skin biopsies for identifying a LPA signature, according to a guided clustering algorithm. The goal of using

this data integration approach was to ensure that the identified gene cluster with high LPA treatment response was also consistently expressed and correlated in skin biopsies.

The identified fibroblast LPA signature was subsequently reduced to a single composite biomarker called pathway activation index (PAI) computed as coefficient of a robust regression on the expression matrix of the LPA signature at each treatment visit (median polish) (15). PAI was then used as a surrogate biomarker for investigating SAR100842 treatment response.

Exploratory endpoints were change from baseline to Week 24 in the mRSS and SHAQ, and also the change in pain or pruritus from baseline to Week 8 and Week 24.

### **Statistical methods**

#### **Sample size determination**

No formal sample size calculation was performed for this proof of biological activity study and the sample size for this study was based upon empirical considerations.

#### **Safety analyses**

The safety analyses were based on the safety population of all randomized patients who actually received at least 1 dose of the IMP, and analyzed according to the treatment actually received in the core part or extension part. The safety analyses were descriptive.

## **Efficacy analyses**

The efficacy analyses were based on the modified intent-to-treat (mITT) population, of all randomized population who actually received at least 1 dose of IMP and with at least 1 post-IMP-administration measurement during the blinded period (double-blind phase). The mITT population for the extension part was defined as randomized population who did actually receive at least 1 dose of IMP in the extension part with at least 1 post-IMP administration measurement during the extension part.

An analysis of covariance (ANCOVA) was performed for the total mRSS score and HAQ-DI on the change from baseline to Week 8 on the mITT population, with treatment group as the main factor and the baseline score centered on its means in the mITT population as a continuous covariate. Student-t test was used to determine the superiority of SAR100842 300 mg BID over placebo at Week 8 with nominal 2-sided type I error rate of 5%.

The analysis of other SHAQ variables was purely descriptive.

All other secondary endpoints were described by treatment and analyzed within an ANCOVA.

## **Biomarker analyses**

The biomarker analyses were based on the population of all randomized and treated patients who received at least 4 weeks of study drug with at least a baseline and a post-baseline assessment. Prior to all statistical analyses, mRNA data were normalized. Each biomarker was analyzed using descriptive statistics. For each of the following skin

biomarkers related to the disease: COMP, THBS1, Collagen 1A1 (COL1A1) mRNAs and  $\alpha$ -SMA labeling (13), the change from baseline to Week 8 measurement was analyzed using a rank ANCOVA, with treatment group as fixed effect and baseline value as covariate.

### **Target engagement**

SSc fibroblasts were prepared from forearm biopsies, following established outgrowth conditions, and cultured in F-12K medium with 10% heat-inactivated fetal calf serum (FCS), 100 units/ml of penicillin, 100 $\mu$ g/ml of streptomycin, and 0.3 mg/ml of L-glutamine. Dermal fibroblasts from healthy volunteers (N=4) and SSc patients (N=10) were seeded and treated with LPA at 10 $\mu$ M for 24h (vs vehicle). Supernatants were removed and cells were rinsed, stored and total RNA was purified using RNeasy Mini kit (Qiagen). The same methodology was used to extract RNA from skin biopsies from patients of the ACT study. Gene expression was measured by whole transcriptome profiling analysis using Affymetrix HG-U133 plus 2.0 GeneChips. From each GeneChip result, a probe cell intensity data file (CEL file) was computed and represented an individual gene expression profile. Samples were clustered based on Euclidean distance and correlation for evaluating the similarity of the quality of each array against the quality of the other arrays. Principal component analysis (PCA) on expression data was performed as well as PCA on quality control metrics of the raw data provided by the Affymetrix platform (R-package simpleaffy).

The guided clustering algorithm (15) was used for the identification a set of genes with high LPA perturbation in the cell culture study and that were consistently expressed in the skin biopsies of patients with SSc. A logistic regression model was computed for each probeset separately, with the LPA treatment label as outcome variable (LPA=1, Placebo=0) and the probeset as independent variable. Each model was adjusted by the fibroblasts type (normal/SSc). The coefficient of the probeset in the model was used as LPA activation

strength for weighting the probesets. The obtained weights were used in conjunction with the expression profile in skin biopsies at baseline to extract the LPA signature. The LPA signature was condensed into one surrogate marker called PAI.

Descriptive statistics of change in LPA PAI from baseline to end of 8-week treatment were computed by treatment arm. The difference between SAR100842 and placebo was investigated using the model:  $\Delta PAI_{main\ part} = \beta_0 + \beta_1 * treatment + \beta_2 * scaled\_baseline PAI + \xi$ .

Targeted gene expression analysis of selected LPA related and fibrosis genes was carried out in the same skin biopsy samples using RT-qPCR.

### **Ethical approval**

The protocol and its amendments were submitted to independent Ethics Committees and/or Institutional Review Boards for review and written approval. All patients provided written Informed consent prior to the conduct of any study-related procedures, and the optional skin biopsy informed consent form (ICF) was obtained from patients who agreed to the collection of skin biopsy.

In addition, dermal fibroblasts were grown from skin biopsies of another cohort of SSc patients fulfilling the ACR/EULAR classification criteria. Procedure was approved by the local ethics committee (University of Naples) and patients signed informed consent forms.

## RESULTS

### Baseline characteristics of the patients

Of 48 patients screened, 16 patients were screen failures (33.3%). Thirty-two (32) patients were randomized into the study: 15 received 300 mg SAR100842 twice a day and 17 received placebo for 8 weeks. Patients across treatment groups had comparable demographic characteristics at baseline consistent with the overall population of dcSSc patients (Table 1).

One patient in the SAR100842 group discontinued treatment on personal request but was included in the mITT analysis. Of the 32 patients initially randomized to the double-blind part, 30 were enrolled into the extension part: Sixteen (16) and 14 patients initially treated with placebo or SAR100842, respectively, participated in the extension part. One patient in the placebo/SAR100842 group and 1 patient in the SAR100842 /SAR100842 group discontinued the treatment due to adverse events, and withdrew upon patients' request.

The mean overall compliance was comparably high among treatment groups (99.6% in the placebo versus 98.5% in the SAR100842 group).

### Safety: SAR100842 showed good tolerability

Overall, SAR100842 was well tolerated: The detailed AE are described in supplementary material (Table S1). A total of 80% patients in the SAR100842 versus 71% patients in the placebo group reported at least one treatment emergent adverse event (TEAE). However, most of the TEAEs were mild to moderate in intensity. There was 1 treatment-emergent serious adverse event (SAE) in the SAR100842 group (syncope) in a patient with a medical

history of syncope in childhood. In the extension phase, two (2) patients reported a treatment-emergent SAE, one in each group. Dyspnea was reported in 1 patient 6 days after switching from placebo to SAR100842, and was considered to be related to the investigational medicinal product, while an infected digital ulcer in another patient was not considered to be drug-related. Two (2) patients prematurely discontinued due to TEAEs, 1 for moderate arthritis in the SAR100842/SAR100842 group and 1 for pruritus, skin discoloration and swelling of face edema in the placebo/SAR100842 group.

With regard to the laboratory safety assessments, no safety concern has emerged from the various laboratory parameters (Table S2, in supplementary documents).

#### **Efficacy: change in mRSS during the controlled and extension phases**

Primary analysis was conducted in mITT population on patients who were under treatment until Week 8. There was a numerically greater decrease without reaching statistical significance in the total mRSS score from baseline in the SAR100842 group compared to placebo (mean change [SD]: -3.57 [4.18] vs -2.76 [4.85]; treatment effect: -1.2; 95% CI [-4.37 to 2.02],  $p=0.46$ ; median change: -4 (Q1:Q3=-5:-1) versus -1.00 (Q1:Q3=-5:0) respectively) (Figure 1).

After 24 weeks of treatment, patients in the SAR100842/SAR100842 group experienced a clinically meaningful decrease in total mRSS score (mean change [SD]: -7.36 [4.24]; median change = -7.50) versus baseline, and a high rate (78.6%) of patients improved by at least 5 points (responder). Patients initially receiving 8 weeks of placebo also demonstrated an improvement in mRSS (mean change [SD]: -7.31 [4.59]; median change from baseline= -7.00) after 24 weeks, with a responder rate of 69.2%.

## **Changes in quality of life during the controlled and extension phases**

There was no statistically significant difference in change of HAQ-DI total score from baseline to Week 8 between SAR100842 and placebo (mean change [SD]: 0.00 [0.33] in placebo and -0.14 [0.30] in SAR100842; treatment effect: -0.1; 95% CI [-0.38 to 0.09]). However, it can be pointed out that the mean absolute difference observed in the SAR100842 group (-0.14) versus baseline reached clinically meaningful level; indeed, an improvement of  $\geq 0.14$  of HAQ-DI is considered to be the minimum clinically important difference in patients with SSc. The improvement seen in the mean HAQ-DI total score was clinically significant from baseline to Week 24 compared to Week 8 in both the placebo/SAR100842 and the SAR100842 /SAR100842 group (mean change [SD]: -0.23 [0.30] and -0.15 [0.33], respectively), and the percentage of patients who decreased by  $\geq -0.14$  on the HAQ-DI total score in the 2 groups were comparable.

## **Effects on pruritus and pain during the controlled and extension phases**

Based on preclinical rationale, LPA receptor antagonists may be effective on pruritus. Interestingly, despite a low baseline value, there was a numerical improvement in SAR100842 versus worsening in the placebo group (mean change [SD]: -0.37 [3.92] versus 0.25 [1.79]) in the severity of pruritus using VAS assessed by patients from baseline to Week 8 (on 0-10 scale). Similarly, a reduced score in pruritus in patients with SAR100842 /SAR100842 versus placebo/SAR100842 treatment was observed (mean change [SD]: -1.38 [2.85] versus -0.84 [1.67]). The severity of pruritus was further decreased in patients with SAR100842 or initially treated with placebo at Week 24 compared to Week 8. The severity of pain using Numerical Pain Scale assessed by patients was low at baseline in the study population. No conclusion could be drawn.



### **Biomarker endpoints: changes did not reach significance for skin fibrosis markers**

There was no statistically significant differential expression of any skin mRNA and protein biomarkers nor blood protein biomarkers between placebo and SAR100842. Alpha SMA and collagen Type 1 were used as fibrosis markers and were not modulated by the treatment (Figure 2). Disease signature was evaluated using either the four-gene biomarker as described by Farina *et al* or a combination of THBS1 and COMP. None of these genes change was correlated with the change in mRSS (Table 2). However, there was a trend for reduction in THBS1, after 8 weeks of SAR100842 compared to placebo although not reaching statistical significance (Figure 3). In addition, MS4A4A gene (a marker of M2 macrophages) was also evaluated. The expression of this marker such as the 2-gene signature (14) were not modulated by SAR100842 and was not correlated with the change in mRSS (not shown).

Global change in gene expression in skin samples at 8 weeks between vehicle and treated patients was evaluated using stringent cut-off for false discovery rate (0.05) or less stringent criteria (0.1). No significant difference was observed in any conditions. Data obtained for cut-off of false discovery rate of  $<0.1$  are presented in Table S3.

### **SAR100842 induced target engagement in LPA pathway**

There was a numerical reduction without reaching statistical significance from baseline of some LPA-pathway biomarkers (PAI-1, Wnt2 and SFRP4) in SAR100842 versus placebo group (Figure 4). Although not significant the decrease of these biomarkers is of interest since they have been shown to be regulated by LPA and SAR100842 in dermal fibroblasts of SSc patients. Thus, a post-hoc analysis was performed to identify a more global LPA signature in dermal SSc fibroblasts and skin biopsies and to evaluate the impact of SAR100842 on this signature in patient skin to assess target engagement.

The signature was identified using both microarray data obtained in SSc dermal fibroblast treated for 24 hours with LPA and microarray data from skin biopsies of SSc patients at baseline. A guided clustering method was performed to give weight to genes that were expressed at significant level following LPA treatment but that were also expressed at a significant level in skin biopsies. This led to a list of 47 genes identified as LPA signature provided in the supplementary document (Table S4). This signature reflects pathways, like proliferation, EGF signaling known to be mechanistically part of LPA responses in other cell types. These genes were reduced to a unique surrogate biomarker in one dimension called PAI using the median polish algorithm (16). The PAI was extracted as row effect as it represents the summary expression in each patient. A significant decrease in PAI was observed in the SAR100842 group (P-value = 0.0089) (Figure 5).

## DISCUSSION

LPA is a phospholipid growth factor targeting cells through a number of cell surface receptors that has been implicated in the pathogenesis of SSc. Of the most interest, it has appeared as a possible mechanism contributing to excessive tissue fibrosis, mainly through LPA<sub>1</sub> receptor activation (17), as observed in SSc. Recent findings further emphasis on the key role of autotaxin, and LPA axis in SSc (18). SAR100842 is a low molecular weight, selective inhibitor of LPA<sub>1</sub> receptor, being developed as a potential novel therapy for SSc with the aim of reducing or even reversing the progression of fibrosis. This phase II study was the first to assess oral administration of SAR100842 in patients with early diffuse SSc.

The safety and tolerability of SAR100842 was the primary outcome and SAR100842 was shown to be well tolerated in patients with dcSSc.

In pre-clinical studies, the administration to rats at doses up to 2000 mg/kg/day caused no toxicologically relevant effects. Compound related findings were limited to a slightly higher incidence of regurgitation in females at high dose and the present study did not show any specific gastro-intestinal adverse events in SSc patients. In previous phase I studies, the safety profile was very good and overall, the most frequently reported related adverse events were headache, symptomatic orthostatic hypotension or postural dizziness, and flatulence. Those adverse events were not severe or serious. In the present study both in the short-term double-blind and the longer-term open part, no safety signal emerged on vital signs, orthostatic hypotension, ECG, or laboratory parameters. A common toxicological concern with anti-fibrotic agents is whether patients may exhibit a delay in normal wound healing. Studies with LPA receptor antagonists have been reassuring using incisional and excisional wounding studies in rats (18), but it is noteworthy that in the present study, despite one third of the patients had digital ulcerations at baseline, no overt safety concerns emerged for them, confirming the good safety profile in SSc patients.

The clinical efficacy of SAR100842 was part of the secondary endpoints but no effect was expected on the mRSS after 8 week of treatment because mRSS is slow in changing. Nevertheless, at the end of the double-blind period, a numerically greater decrease in total mRSS score from baseline in the SAR100842 group compared to placebo was detected without reaching statistical significance (treatment effect: -1.2; 95% CI [-4.37 to 2.02]; median change SAR100842 versus placebo: -4.00 versus -1.00, respectively). Also, there was a numerical greater reduction without reaching statistical significance in the HAQ-DI (treatment effect: -0.1; 95% CI [-0.38 to 0.09]) in the SAR100842 group versus placebo. These findings are promising; they might be due to the mechanism of action of SAR100842 and/or may also be explained by a large proportion of subjects on background immunosuppressive medications. These findings were supportive of the effect observed

after 24 weeks of treatment where patients experienced a clinically meaningful decrease in total mRSS score (median change= -7.5) with a high responder rate of 78.6% for patients improved by at least 5 points (19) versus baseline and similar benefit was observed in the HAQ-DI. Although being secondary end-points and with weak statistical power, the size of the decrease must be underlined and is larger than that observed in other trials targeting the same SSc population. Furthermore, the similar trend observed for skin changes and quality of life is encouraging and promising for future trials. Nevertheless, and despite being encouraging, the open label data should be interpreted with caution.

This study must be interpreted taking into account its limitations. The sample size was not large, but in line with the design of a proof-of biological activity study looking primarily at safety. The duration may be considered as short and most trials are expected to last more than 6 months but the observed changes of mRSS in this population are promising. SSc is a systemic disease and organ involvement defines the prognosis. No data could be provided on organ involvement from the present study and this will have to be addressed in the future.

Pharmacodynamic and biomarker assessments were part of the secondary endpoints. There was no statistically significant differential expression of any biomarker between the 2 groups of patients. The response rate was higher than expected (20%) in the placebo group, while the response rate in the SAR100842 group was in the initial assumption range (60%).

Using a new unbiased statistical analysis, a guided clustering algorithm allowed the identification of a set of genes with high LPA perturbation in the cell culture study and that were consistently expressed and correlated with similar expression in skin samples from patients. This LPA signature was then reduced to one dimension and change in the resulting

PAI was computed in skin biopsies of patients treated with placebo vs patients treated with SAR100842. A significant effect of SAR100842 on change from baseline for PAI was indicative of an effect of SAR100842 on the LPA signature (15 patients per treatment arm) demonstrating target engagement upon SAR100842 treatment for 8 weeks.

The optimal clinical trial duration for patients with SSc is still unknown. Some observations regarding collagen metabolism suggest that a clinical trial duration of 24 weeks or longer might be recommended. Indeed, in the phase 2 tocilizumab data (20), the 2-gene biomarker was able to differentiate TCZ from placebo at 24 weeks. The biomarkers in clinical trials of SSc have shown to correlate with skin fibrosis (as seen here) rather than predict skin progression. In addition, the collagen turnover (which is a product of collagen production and collagen degradation) may require several weeks to be modulated and this also depends whether the pharmacologic agent directly (e.g. Anti-TGF-beta inhibitor) or indirectly (current inhibitor) target collagen products. A longer trial with clinical and biological outcome measures at 4-6 months may have shown statistically significant differences in this trial.

This study demonstrates that LPA1 blockade by SAR100842 is well tolerated in early dcSSc patients. The results show target engagement with SAR100842 and some promising clinical and biological changes. Nevertheless, skin fibrotic biomarkers cannot infer treatment effect but may be informative as shown in other recent trials (20-22). Altogether these results suggest the potential clinical benefit of SAR100842 in dcSSc patients for whom unmet needs remain (23) and deserve its evaluation in confirmatory trials.

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### Legends of the figures:

Figure 1: Box plot for mRSS change from baseline to Week 8 on mITT population.

Figure 2: Boxplots for Skin fibrosis markers (changes from baseline to week 8)

Figure 3: Boxplots for Skin fibrosis 4 gene-biomarkers (changes from baseline to week 8)

Figure 4: Boxplots for LPA pathway markers (changes from baseline to week 8)

Figure 5: Boxplots of change in Pathway Activation Index (PAI) from baseline to EOT Week 8 by treatment groups

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Table 1: Baseline characteristics

	Placebo (n=17)	SAR100842 (n=15)	All (n=32)
Age (years): mean (SD)	50.6 (11.3)	48.8 (10.3)	49.8 (10.7)
Sex: n (%) female	12 (71%)	9 (60%)	21 (66%)
Race: n (%) Caucasian/white	13 (76%)	13 (87%)	26 (81%)
Weight (kg): mean (SD)	70.6 (16.8)	75.1 (19.3)	72.7 (17.9)
Smokers: current n (%)	3 (18%)	2 (13%)	5 (16%)
Disease duration (months): mean (SD)	19.6 (7.4)	20.4 (8.9)	20.0 (8.0)
Raynaud's phenomenon: n (%)	17 (100%)	14 (93%)	31 (97%)
Digital ulcers (past or current): n (%)	6 (35%)	4 (27%)	10 (31%)
Joint synovitis: n (%)	5 (29%)	4 (27%)	9 (28%)
Tendon friction rubs: n (%)	6 (35%)	7 (47%)	13 (41%)
Renal crisis: n (%)	1 (6%)	0	1 (3%)
Dyspnoea (significant) : n (%)	7 (41%)	2 (13%)	9 (28%)
Fibrosis on plain x-ray: n (%)	3 (18%)	1 (7%)	4 (13%)
Positive anti-centromere abs: n (%)	1 (6%)	0	1 (3%)
Positive anti-Scl70 abs: n (%)	5 (29%)	4 (27%)	9 (28%)
Positive anti-RNA pol III abs: n (%)	4 (24%)	8 (53%)	12 (38%)
Baseline mRSS:			
mean (SD)	24.8 (7.8)	22.7 (8.2)	23.8 (7.9)
Median: min-max	23 (15-38)	21 (15-44)	22 (15-44)
Baseline HAQ-DI:			
mean (SD)	1.27 (0.75)	1.23 (0.77)	1.25 (0.75)
Median: min-max	1.25 (0.0-2.5)	1.38 (0.0-2.4)	1.37 (0.0-2.5)
Any prior immunosuppressive or steroid medications	14 (82%)	10 (67%)	24 (75%)
Mycophenolate mofetil	5 (29%)	7 (47%)	12 (38%)
Methotrexate	9 (53%)	1 (7%)	10 (31%)
Systemic steroids	8 (47%)	6 (40%)	14 (44%)
Topical steroids	1 (6%)	1 (7%)	2 (6%)

Table 2: Absence of correlation between change in 4 genes biomarkers and change in mRSS

GENE	Corr.ch.gene_ch.mRSS	Correlation.Pvalue
COMP	0.011	0.96
TSP1	0.0074	0.97
SIGLEC1	0.043	0.82
IFF44	-0.031	0.87





